U30031PCT
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Patent claims

1. Method for producing a heterodimeric specific wild type- or chimeric T-cell receptor (TCR), containing a first chain and a second chain that interact one with another at at least one surface, wherein the at least one surface is subjected to a rational mutagenesis, such that the at least one surface of the first chain or the surface of the second chain comprises a sterically projecting group, which interacts with a sterically recessed group on the at least one surface of the corresponding first chain or second chain, comprising the steps of:
   (a) providing the DNA-molecules, comprising the coding regions for the at least one surface to be mutated of the first chain or second chain, in (a) joint or separate mutagenesis-vector system(s),
   (b) mutagenesis of the DNA-molecules in a manner known as such, wherein the nucleic acid sequence encoding for the at least one surface is modified compared to the initial sequence in such a manner that
   in the at least one surface of the first chain or the at least one surface of the second chain, a sterically projecting group is introduced, and
   in the corresponding at least one interacting surface of the second chain or the first chain, a sterically recessed group is introduced, whereby individual mutated fragments are produced, and
   c) translation of at least two of the single mutated fragments from step b), such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least one surface is selectively promoted, and
   d) presentation of the heterodimeric first-chain/second-chain TCR by a T-cell.

2. Method according to claim 1, wherein step c) is replaced by the following steps:
   (c') optionally, sub-cloning of the mutated fragments into a suitable transfection-vector system,
   (c'') transfection or co-transfection or transduction of at least two of the mutated fragments into a mutant TCR-deficient T-cell, and
   (c''') expression of the heterodimeric first-chain/second-chain TCR in a recombinant T-cell.

3. Method according to claim 1, wherein step c) is replaced by the following steps:
   c') *In vitro*-translation or *in vivo*-translation of at least two of the individual mutant-fragments from step b) and, optionally, subsequent isolation and/or purification of the translated mutant-fragments,
   such that the pairing of the heterodimeric specific first-chain/second-chain TCR being mutated at least on one surface is selectively promoted, and
   c'') introduction of the mutated specific first-chain/second-chain TCR into a T-cell.

4. Method according to claim 3, wherein the *in vivo* translation takes place in a host cell.

5. Method according to claim 3 or 4, wherein the introduction takes place by liposome-transfer.

6. Method according to any of claims 1 to 5, wherein the TCR is an alpha/beta TCR, gamma/delta TCR, a humanized or partially humanized TCR, a TCR being provided with additional (functional) domains, a TCR being provided with alternative domains, e.g. a TCR being provided with a different transmembrane domain as a membrane anchor.

7. Method according to any of claims 1 to 6, wherein the amino acid as introduced after the mutagenesis of the DNA-molecules are further suitably chemically modified, in order to thereby introduce a sterically projecting group or a sterically recessing group.

8. Method according to any of claims 1 to 6, wherein the amino acid as introduced after the mutagenesis of the DNA-molecules directly provides the sterically projecting group or the sterically recessing group.

9. Method according to any of claims 1 to 8, wherein the amino acids as introduced by the mutagenesis of the DNA-molecules are chosen in such a manner that a mutual exchange of the amino acids on the surfaces of the interacting chains of the TCR is achieved.

10. Method according to any of claims 1 to 9, wherein the amino acid that has been introduced after the mutagenesis of the DNA-molecules that introduces a sterically recessing group compared to the initial sequence is selected from glycine, serine, threonine, valine and alanine.

11. Method according to any of claims 1 to 9, wherein the amino acid that has been introduced after the mutagenesis of the DNA-molecules that introduces a sterically projecting group compared to the initial sequence is selected from tryptophane, lysine, arginine, phenylalanine, cysteine and tyrosine.

12. Method according to any of claims 1 to 11, wherein at least two surfaces of a TCR-chain are simultaneously subjected to mutagenesis.

13. Method according to any of claims 1 to 12, wherein the corresponding interacting surfaces are localized in the variable domains of the TCR-chains.

14. Method according to any of claims 1 to 13, wherein the corresponding interacting surfaces are localized in the constant domains of the TCR-chains.

15. Method according to any of claims 1 to 14, wherein the domains of the TCR-chains to be mutated are selected from mammalian, in particular human and/or mouse-domains.

16. Method according to any of claims 1 to 15, wherein the rational mutagenesis of the TCR-chains at the same time leads to a humanization of the TCR.

17. Method according to any of the preceding claims, wherein the alpha- and beta-chains of an MDM2(81-88)-specific TCR are used as alpha-chain and beta-chain, and wherein the Gly192 of the constant region of the alpha-chain and the Arg208 of the constant region of the beta-chain are exchanged by Arg 192 in the constant region of the alpha-chain and by Gly208 in the constant region of the beta-chain.

18. Method according to claim 17, wherein simultaneously with or subsequent to the exchanges at positions 192 and 208, additional positions are modified in the chains.

19. Method according to any of claims 1 to 18, wherein a retroviral vector, in particular pBullet, is used as a transfection system.

20. Mutated alpha- or beta-chain of a TCR, produced according to a method according to any of claims 1 to 19.

21. Mutated TCR, in particular mutated MDM2(81-88)-specific TCR (Seq ID No. 2 and Seq ID No. 5), comprising at least one mutated alpha- and beta-chain according to claim 20.

22. Isolated nucleic acid, comprising a sequence coding for a mutated alpha- or beta-chain of a TCR according to claim 20.

23. DNA- or RNA-vector molecule, comprising at least one or several nucleic acid(s) according to claim 22, and that can be expressed in cells.

24. Host cell containing a DNA- or RNA-vector molecule according to claim 19.

25. Recombinant T-cell, expressing at least one mutated TCR according to claim 21.

26. Composition, comprising a recombinant T-cell according to claim 25.

27. Use of a mutated alpha- or beta-chain of a TCR according to claim 20, of a mutated TCR according to claim 21 and/or a recombinant T-cell according to claim 25 for the production of therapeutics and/or prophylactics for the treatment of cancerous diseases.

28. Use according to claim 27, wherein a cancerous disease is treated that is in connection with a modified expression of MDM2, p53, Her-2/neu, Ras, tyrosinase, MART, Gp100, MAGE, BAGE, MUC-1, CD45, CD19 or PRDI-BF1.